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Circulation. 2009;119:1747-1757; originally published online March 23, 2009;
doi: 10.1161/CIRCULATIONAHA.108.800938

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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Stress Doppler Echocardiography in Relatives of Patients With Idiopathic and Familial Pulmonary Arterial Hypertension

Results of a Multicenter European Analysis of Pulmonary Artery Pressure Response to Exercise and Hypoxia

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Background—This large, prospective, multicentric study was performed to analyze the distribution of tricuspid regurgitation velocity (TRV) values during exercise and hypoxia in relatives of patients with idiopathic and familial pulmonary arterial hypertension (PAH) and in healthy control subjects. We tested the hypothesis that relatives of idiopathic/familial PAH patients display an enhanced frequency of hypertensive TRV response to stress and that this response is associated with mutations in the bone morphogenetic protein receptor II (*BMPR2*) gene.

Methods and Results—TRV was estimated by Doppler echocardiography during supine bicycle exercise in normoxia and during 120 minutes of normobaric hypoxia ($\text{FiO}_2=12\%$; ≈ 4500 m) in 291 relatives of 109 PAH patients and in 191 age-matched control subjects. Mean maximal TRVs were significantly higher in PAH relatives during both exercise and hypoxia. During exercise, 10% of control subjects but 31.6% of relatives ($P<0.0001$) exceeded the 90% quantile of mean maximal TRV seen in control subjects. Hypoxia revealed hypertensive TRV in 26% of relatives ($P=0.0029$). Among control subjects, TRV at rest was not related to age, sex, body mass index, systemic blood pressure, smoking status, or heart rate. Within kindreds identified as harboring deleterious mutations of the *BMPR2* gene, a hypertensive TRV response occurred significantly more often compared with those without detected mutations.

Conclusions—Pulmonary hypertensive response to exercise and hypoxia in idiopathic/familial PAH relatives appears as a genetic trait with familial clustering, being correlated to but not caused by a *BMPR2* mutation. The suitability of this trait to predict manifest PAH development should be addressed in long-term follow-up studies. (*Circulation*. 2009;119:1747-1757.)

Key Words: echocardiography ■ echocardiography, stress ■ genetics ■ hypertension, pulmonary ■ hypoxia ■ pulmonary heart disease

Idiopathic (I) or familial (F) pulmonary arterial hypertension (PAH) is chronically progressive and believed to evolve slowly, with an asymptomatic increase in pulmonary arterial constriction and remodeling over several years.^{1,2} In most patients, the disease is not diagnosed until pulmonary artery pressure is markedly elevated and right ventricular

dysfunction has begun.³ The results of the Endothelin Antagonist trial in mild symptomatic PAH (EARLY) indicate that diagnosis and treatment of PAH even a few months earlier might improve time to clinical worsening and emphasize that PAH needs to be diagnosed and treated in the early stages.⁴ Because mutations of the bone morphogenetic pro-

Received June 26, 2008; accepted January 23, 2009.

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Clinical trial registration information—URL: <http://cordis.europa.eu/fp5/home.html>.

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Circulation is available at <http://circ.ahajournals.org>

DOI: 10.1161/CIRCULATIONAHA.108.800938

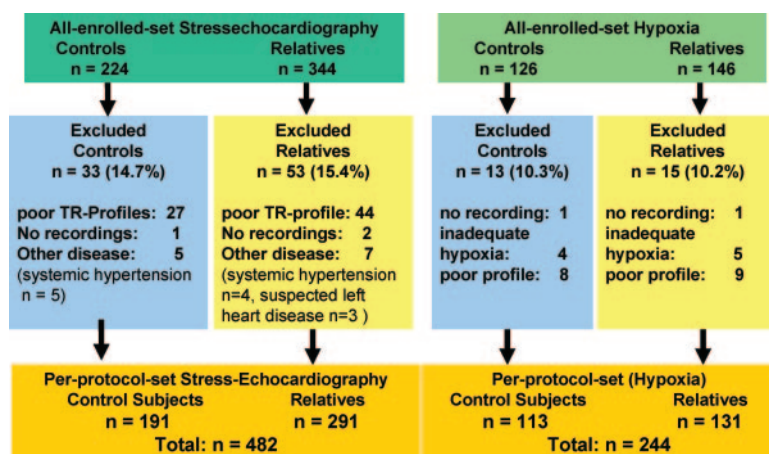


Figure 1. Study population. Left, healthy control subjects and relatives who performed supine bicycle exercise; right, subjects assessed during 120 minutes of hypoxia.

tein receptor II (*BMPT2*) have been identified in patients with I/FPAH,^{5–7} noninvasive methods aiming at the identification of persons at risk for the disease have gained attention. In a previous study, family members of FPAH patients who shared the risk haplotype with the index patient showed an increased pulmonary artery systolic pressure (PASP) rise during exercise as assessed by echocardiography.^{8,9} These results suggest that asymptomatic gene carriers, in the absence of manifest pulmonary hypertension, might be identified by their enhanced PASP response to supine bicycle exercise.⁸

Clinical Perspective p 1757

Most pulmonary hypertension (PH) guidelines recommend a screening by echocardiography at rest for patients at risk of PAH, including relatives of PAH patients and patients with systemic sclerosis.¹⁰ Although echocardiography at rest has been established in the diagnosis of manifest PH,¹¹ it has rarely been evaluated in at-risk groups. Furthermore, echocardiography at rest has been shown to have a low sensitivity and specificity in subjects in whom PAH is not clinically evident. Echocardiography during exercise may be a more promising approach to detect early stages of PAH. However, the feasibility of using stress Doppler echocardiography for this indication has not been assessed. Another problem is that no confirmed consensus exists as to which PASP threshold is diagnostically relevant for exercise-induced PH, particularly if stress echocardiography is applied. A high threshold would increase specificity but decrease sensitivity.¹² Only a few invasive and noninvasive studies have analyzed the normal values for pulmonary artery pressures during exercise and prolonged hypoxia. Gurtner et al¹³ and Janosi et al¹⁴ showed that in healthy subjects the systolic pressures do not exceed 40 mm Hg even during heavy exercise. Bossone et al¹⁵ found higher values in athletes as a consequence of increased flow and left atrial pressure. Nevertheless, even in athletes, PASP did not exceed 40 mm Hg until high workloads (>160 W) had been reached.¹⁵

Beside stress Doppler echocardiography, assessment of tricuspid regurgitation velocity (TRV) during hypoxia may be another interesting noninvasive screening tool for early PAH manifestation. In previous studies, subjects susceptible to high-altitude pulmonary edema displayed a hypertensive PASP increase not only during hypoxia^{16,17} but also during exercise in

normoxia.^{18,19} Thus, a hypertensive increase in pulmonary artery pressure during exercise and hypoxia may be linked.

In the present multicentric study, we tested the hypothesis that relatives of I/FPAH patients display an enhanced frequency of a hypertensive rise in pulmonary artery pressure in response to exercise and hypoxia compared with unrelated healthy control subjects. To perform this test, threshold values of “normal” and “hypertensive” TRV values under conditions of exercise and hypoxia were assessed in the healthy control group by predefined criteria. Moreover, in the relatives of I/FPAH patients with known *BMPT2* mutation, we asked whether such hypertensive pulmonary artery pressure response to exercise and hypoxia might be linked to the presence of this mutation.

This is the first large-scale multicenter study of Doppler stress echocardiography in pulmonary hypertension. A long-standing European tradition exists of multicenter effectiveness in stress echocardiography studies in coronary artery disease and in dilated cardiomyopathy.^{20,21} Our study might extend the basis for incorporation of PH in the indications for stress echocardiography.

Methods

Study Population and Design

We assessed relatives ≥ 12 years of age of unrelated adult index patients with manifest idiopathic or familial PAH who did not have any intervening cardiac or pulmonary disease (Figure 1). In all index patients, the diagnosis was established at the PH-specialized centers according to current guidelines.^{1,10,11} All patients underwent a detailed clinical examination, including right heart catheterization. Testing for vasoreactivity and left heart catheterization were performed when clinically indicated. The control group consisted of age- and sex-matched subjects who were recruited from the employees of the participating hospitals and from students of medical and physiotherapy schools. Age and sex matching was performed in each study center as a group matching. Therefore, clinical assessment of family members and control subjects included a medical history, physical examination, 12-lead ECG, echocardiography at rest and during supine bicycle exercise at normoxia, and echocardiography during 120 minutes of normobaric hypoxia ($\text{FiO}_2 = 12\%$). In relatives or control subjects with hypertensive PASP, secondary reasons were excluded by a cascade of clinical examinations, including echocardiography of the left heart, chest x-ray, pulmonary function tests, and measurement of arterial blood gases. Acute or chronic pulmonary and cardiac diseases were ruled out in all family members and control subjects. Blood samples were collected for genetic analysis

from all patients and relatives. The protocol of this study was approved by the ethics committees of the participating universities, and the participants or their parents gave written informed consent.

Echocardiography

Two-dimensional and color-flow guided continuous-wave Doppler echocardiographic recordings were performed by experienced cardiac sonographers using 2.5-MHz duplex probes and conventional equipment (HP [Palo Alto, Calif] Sonos 2000, Acuson Sequoia C512, HP Sonos 4500, GE [Tampa, Fla] Vivid 7, Aloka SSD-5500, and Aloka SSD-2200) (1) at rest and during stress echocardiography in normoxia and (2) during baseline in normoxia and at 45, 90, and 120 minutes of hypoxia ($FiO_2=12\%$). PASP was estimated from peak tricuspid regurgitation jet velocities according to the modified Bernoulli equation: $PASP=4(V)^2+RAP$, where V is the peak velocity (in m/s) of tricuspid valve regurgitant jet (TRV) and RAP is right atrial pressure. In the absence of obstruction to right ventricular outflow, right ventricular systolic pressure equals PASP.²² For all calculations, the mean value of at least 3 TRV measurements was used. Right atrial pressure was estimated from characteristics of the inferior vena cava.²³ If it was <20 mm in diameter and decreased during inspiration, we add 5 mm Hg, which was the case in all subjects. Left ventricular and left atrial size, left ventricular ejection fraction, and diameters of the left ventricular wall were obtained in all subjects. Subjects with abnormal values for left ventricular size, septal thickness (>12 mm), and left ventricular ejection fraction were excluded. Diastolic left ventricular function was evaluated with pulsed-wave Doppler in the mitral and tricuspid inflow measuring E/A and E/e'. Subjects with suspected diastolic dysfunction had to be excluded.

Stress Doppler Echocardiography

Relatives and control subjects underwent a standardized echocardiography Doppler bicycle exercise on a variable-load supine bicycle ergometer (ER 900 EL, Ergoline, Bitz, Germany) as described previously.⁸ Workload was increased by 25 W every 2 minutes to an exercise capacity or symptom-limited maximum. TRV, heart rate, and systemic blood pressure were analyzed at each stage. Echocardiographic assessment was continuously recorded on videotape and stored in DICOM format for further offline analysis.

Measurements During Hypoxia

Measurements during hypoxia were performed in 3 of the 6 participating centers with a gas mixture of 12% oxygen and 88% nitrogen corresponding to an altitude of 4500 m. In Heidelberg, measurements were performed in a hypoxic chamber; in Brussels and Giessen, a facial mask was used. The subjects were examined in the supine position, and oxygen saturation and heart rate were recorded continuously with a fingertip pulse oximeter (Ohmeda Biox 3700, Ohmeda, Louisville, Colo).

Genetic Analysis

BMPR2 mutation analysis was performed in all index patients and relatives with denaturing high-performance liquid chromatography and cycle sequencing with Big Dye terminators (version 1.1, Applied Biosystems, Foster City, Calif) on an ABI 3700 sequencer as described previously.^{7,9}

Quality Control

Echocardiography measurements and genetic analyses were subjected to strict quality control. Great attempts were made to obtain high-quality TRV profiles and reliable genetic data. All participating centers used the same supine bicycle tables and protocols for exercise and hypoxia. At the start of this project, experienced echocardiographic examiners at all centers were trained in the applied techniques. Recordings were stored in DICOM format and on videotape and were centrally reanalyzed offline in random order and in a blinded fashion. The offline readings were used for calculations. The sonographers who assessed TRV had no knowledge of the genetic data. *BMPR2* mutation screening was performed in 2 independent Institutes of Human Genetics in London and

Heidelberg with crossover analysis for quality control. Genetic investigators were blinded to clinical data.

Statistical Analysis

Data in figures and tables are given as mean \pm SD. All reported values of TRV represent the mean of at least 3 measurements. Baseline characteristics of relatives and control subjects were compared by 2-tailed Student's t test. For comparison of categorical variables, the χ^2 test was performed. The comparisons of groups for TRV at rest and during exercise and hypoxia were performed by ANOVA. These tests were adjusted for centers and familial relations. Therefore, the mean values obtained in each family and center were used as covariates. For descriptive analysis of TRV values at rest and during exercise and hypoxia, histograms, Q-Q plots, and the method of kernel density estimation²⁴ were used. Correlation coefficients adjusted for familial relationships were used to describe TRV measurements during exercise and hypoxia. For the comparisons of baseline with exercise and baseline with hypoxia, paired t tests were performed. Comparisons of the proportion of family members with exaggerated TRV response between *BMPR2*-positive and *BMPR2*-negative subjects were performed by with Proc Genmod, SAS version 9.1 (SAS Institute, Inc, Cary, NC), with the logistic link function taking into account clustered measurement per family. Comparisons of TRV values at exercise and hypoxia were adjusted for multiple comparisons. Values of $P<0.025$ remained significant. Unadjusted values of $P<0.05$ were considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Study Population

In total, 122 index patients, 344 relatives, and 224 unrelated control subjects were recruited. Thirteen index patients were excluded either for lack of relatives assessed clinically or for lack of blood samples. Thirty-three control subjects (14.7%) and 53 relatives (15.4%) had to be excluded because of inadequate Doppler signals during exercise or as a result of a diagnosis of diseases likely affecting pulmonary artery pressure such as systemic arterial hypertension and diastolic left ventricular dysfunction (Figure 1). Thus, the final study group performing stress echocardiography included 482 subjects, 191 unrelated control subjects, and 291 family members from 109 unrelated index patients recruited in 6 centers from 5 European countries (Figure 1, Table 1). In the group assessed during hypoxia, 13 control subjects (10.3%) and 15 relatives (10.2%) had to be excluded because of inadequate Doppler profiles and/or inadequate hypoxemia. In 1 relative and 3 control subjects, the assessment had to be terminated after 60 minutes of hypoxia because of nausea, headache, or low O_2 saturation (SaO_2 , 38%), respectively. Thus, the final study group included 244 subjects, 113 control subjects, and 131 relatives who completed 120 minutes of hypoxia (Figure 1). Relatives and control subjects had similar demographic data, blood pressures, heart rates, and proportion of smokers (Table 2). Hemoglobin values, left ventricular size, left ventricular systolic and diastolic function, and the diameter of the left atrium (mean left atrial diameter, 29.29 ± 5.27 mm in control subjects and 29.28 ± 5.5 mm in family members) were normal in all control subjects and family members. Mitral valve prolapse without regurgitation was seen in 21 family members (9.9%) and in 7 control subjects (4.3%). Five family members (2.4%) and 1 control subject (0.6%) had a very mild

Table 1. Study Population of Each Center

	Centers, n						Total
	Bologna	Brussels	Warsaw	Clamart	Giessen	Heidelberg	
Index patients	10	10	19	19	26	25	109
Relatives	12	35	56	55	67	66	291
Control subjects	10	36	25	30	23	67	191

thickening of the aortic valve; in 3 family members (1.4%) and 1 control subject (0.6%), a mild aortic regurgitation was detected. Because the changes were very mild, we did not exclude these subjects.

Distribution of TRV at Rest and During Exercise and Hypoxia in Control Subjects and Relatives

TRV at rest showed a normal distribution in the control subjects (Figure 2A and Figure 3B). Family members and control subjects reached similar levels during exercise testing (peak heart rate: family members, 146 ± 22 bpm; control subjects, 155 ± 19 bpm; peak $\dot{V}O_2$: family members, 1.8 ± 0.6 L/min; control subjects, 2.2 ± 0.5 L/min). Normal distribution of TRV values was not found during exercise and after 120 minutes of hypoxia, at which point a takeoff pattern beginning at ≈ 3.08 m/s was observed. This is due to a subgroup of control subjects with a hypertensive TRV response to exercise and hypoxia (Figure 2B). The subgroup of control subjects with higher TRV during exercise and/or hypoxia caused a separate peak in the distribution of values (analyzed by the descriptive kernel density estimation of TRV) at ≈ 3.5 m/s, whereas the majority of control subjects had a peak TRV during exercise or hypoxia at ≈ 2.8 m/s (Figure 3C and 3D). This separate second peak of TRV was seen during exercise and hypoxia even when corrected for center effects (Figure 3A). Among the group of relatives, a significantly larger subgroup of subjects was found with TRV values >3.08 m/s during exercise and hypoxia than in the control group, causing a second peak in the distribution at ≈ 3.5 m/s (Figure 3F and 3G). This separate peak was seen in all centers (not shown).

Threshold of Maximal TRV in Controls

The cutoff TRV value discriminating between control subjects showing a normal distribution of values (normal re-

sponse [NR]) and those with hypertensive response to exercise and/or hypoxia (HR) was predefined by the 90% quantile of maximal TRV in control subjects. Applying this criterion, we obtained a cutoff TRV of 3.08 m/s. Thus, control subjects and relatives with a TRV >3.08 m/s (corresponding to PASP values >43 mm Hg) during low-dose exercise (up to 125 W) and/or during 120 minutes of hypoxia were characterized as subjects with hypertensive pulmonary artery pressure response (HR).

Comparison of TRV During Exercise and Hypoxia Between Relatives and Control Subjects

At rest, mean TRV and mean PASP did not differ between relatives (20.7 ± 5.4 mm Hg) and control subjects (20.4 ± 5.3 mm Hg; $P=0.45$; Tables 3 and 4). During exercise, mean maximal TRV was significantly higher in relatives than in control subjects ($P=0.0001$), although control subjects reached a higher mean maximal workload (148 ± 44 W) than relatives (124 ± 42 W). In addition, during hypoxia, mean maximal TRV was significantly higher in relatives than in control subjects ($P=0.0001$). The generalized estimating approach was used to control for center effects. These differences were not related to age or sex. These statistical tests between groups were corrected for centers and familial relation; the mean values for each family were used for calculation. During exercise, 31.6% of relatives but only 10% of control subjects had TRV values >3.08 m/s (Figure 4). During hypoxia, 26% of relatives had values >3.08 m/s compared with 10% of control subjects (Figure 4).

PASP Changes During Exercise and During Hypoxia

During exercise and hypoxia, mean PASP increased significantly more in relatives compared with control subjects

Table 2. Characteristics of Relatives and Control Subjects

Characteristics	Subjects Assessed by Stress Echocardiography per Protocol Set		Subjects Assessed During Hypoxia per Protocol Set	
	Relatives (n=291)	Controls (n=191)	Relatives (n=131)	Controls (n=113)
Female sex, %	43	48	46	40
Age, y	37 ± 16	32 ± 10	36 ± 16	31 ± 11
Height, cm	169 ± 9	173 ± 9	170 ± 10	173 ± 10
Weight, kg	69 ± 15	68 ± 12	70 ± 15	67 ± 12
SBP, mm Hg	125 ± 20	122 ± 14	126 ± 22	122 ± 15
DBP, mm Hg	78 ± 14	77 ± 11	77 ± 15	75 ± 11
Heart frequency, bpm	76 ± 13	74 ± 13	74 ± 12	72 ± 12
Smoker, %	22	16	22	20

SBP indicates systolic blood pressure; DBP, diastolic blood pressure. Data are mean \pm SD unless otherwise indicated.

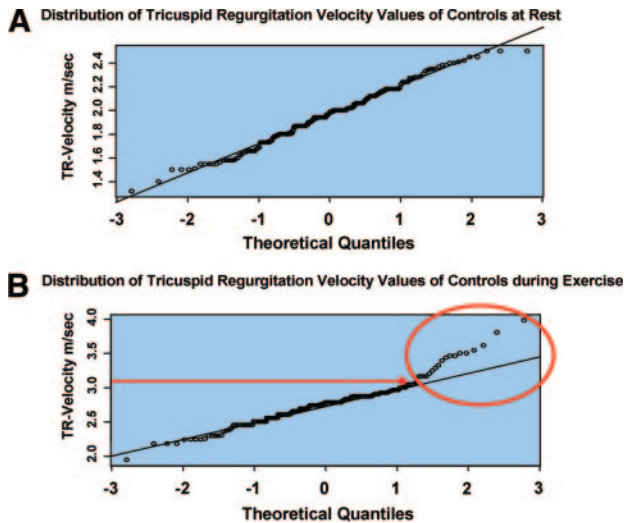


Figure 2. A Q-Q plot with the distribution of TRV values among healthy control subjects (A) at rest and (B) during exercise. A, All TRV values obtained at rest range on a straight line as expected in a normal distribution of values. B, During exercise, TRV values display a deviation of normal distribution as a result of a proportion of subjects with higher TRV values.

(Table 4). Mean PASP during exercise increased from 20.7 ± 5.4 to 39.5 ± 5.6 mm Hg (Δ PASP, 18.8 ± 10.6 mm Hg) in relatives and from 20.4 ± 5.3 to 35.5 ± 5.4 mm Hg (Δ PASP, 15.2 ± 7.1 mm Hg; $P=0.0001$) in control subjects. The same effect was observed during 120 minutes of hypoxia; PASP rose from 20.7 ± 5.4 to 38.4 ± 5.7 mm Hg (Δ PASP, 19.7 ± 9.5 mm Hg) in relatives and from 20.4 ± 5.3 to 33.7 ± 5.5 mm Hg (Δ PASP, 14.4 ± 8.1 mm Hg; $P=0.0029$) in control subjects. The maximal TRV values were found between 90 and 120 minutes of hypoxia. Mean oxygen saturation after 60, 90, and 120 minutes of hypoxia was similar in the subjects assessed in Brussels, Giessen, and Heidelberg, although different methods for hypoxic testing were used (facial mask and hypoxic chamber; Table 3). Maximal PASP of relatives during exercise ranged between 17 and 77 mm Hg; in control subjects, it ranged between 20 and 68 mm Hg.

Comparison of PASP in HR and NR Subjects of Relatives and Control Subjects

HR and NR subjects among the relatives had similar TRV and PASP values at rest. During exercise, mean PASP increased to significantly higher levels in HR relatives (Δ PASP, 28.8 ± 9.1 mm Hg) than in NR relatives (Δ PASP, 12.7 ± 6.2 mm Hg). During exercise and hypoxia, a 2- to 3-fold greater increase in mean PASP was found in the HR compared with the NR subjects. This increase was already evident at low workloads between 50 and 125 W and/or within the first 45 minutes of hypoxia and reached a maximum between 90 to 120 minutes of hypoxia (Δ PASP, 30.4 ± 10.3 mm Hg in HR versus 15.8 ± 5.3 mm Hg in NR). These increases in TRV and PASP during exercise and hypoxia were similar in the HR subjects among both the relatives and control subjects.

Identification of Subjects With Hypertensive TRV During Exercise and Hypoxia

When we analyzed all subjects who completed both exercise and hypoxia assessments (111 control subjects, 130 relatives), we found that during exercise TRV values exceeded 3.08 m/s in 10 control subjects and in 43 relatives. TRV values exceeded 3.08 m/s in 7 of the 10 HR control subjects during exercise and during hypoxia, in 3 of these control subjects during exercise only, and in 4 control subjects during hypoxia only (Figure 5). In contrast, 101 control subjects had TRV values <3.08 m/s during exercise: 97 during exercise and hypoxia and 4 during exercise only. Thus, the positive predictive value for identifying HR subjects among control subjects using supine bicycle exercise and an upper limit of normal TRV of 3.08 m/s was 64% (95% confidence interval [CI], 30.1 to 89.1), and the negative predictive value was 97% (95% CI, 91.5 to 99.4). Within the group of relatives, the positive predictive value was 60.5% (95% CI, 22.3 to 89.1), and the negative predictive value was 91.1% (95% CI, 95.8 to 95), both adjusted for familial relations.

Correlation of TRV With Baseline Variables in Controls

Mean TRV values at rest and during exercise and hypoxia in control subjects did not differ significantly between men and women and between smokers and nonsmokers and were not significantly related to age, sex, systolic or diastolic blood pressure, heart rate, and body mass index (Figure 6). In control subjects, a significant but weak correlation was found of TRV at rest with values during exercise ($r=0.27$, $P=0.0002$; Figure 6Af) and during hypoxia ($r=0.43$, $P=0.0087$; not shown).

Correlation of TRV With Baseline Variables in Relatives

TRV values at rest did not correlate with body mass index or systolic or diastolic blood pressure but did correlate with age ($r=0.13$, $P=0.03$). TRV during exercise in relatives was weak but significantly correlated with body mass index ($r=0.16$, $P=0.0029$), age ($r=0.28$, $P<0.0001$), systolic blood pressure ($r=0.223$, $P=0.0001$), diastolic blood pressure ($r=0.12$, $P=0.0379$), and TRV at rest ($r=0.17$, $P=0.0043$). Relatives with higher values on these parameters generally also showed higher TRV. TRV during hypoxia was weak but significantly correlated with body mass index ($r=0.19$, $P=0.0343$), age ($r=0.38$, $P<0.0001$), systolic blood pressure ($r=0.21$, $P=0.0128$), TRV at rest ($r=0.39$, $P<0.0001$), and TRV during exercise ($r=0.47$, $P<0.0001$).

Genetic Analysis

All P values have been adjusted for familial clustering. Genetic analysis was performed in 109 of 122 recruited index patients with I/FPAH. Of these 109 index patients, 31 had a family history of PAH and were classified as FPAH; 78 were classified as IPAH. In 35 of the 109 index patients and in 16 family members of these 35 patients, a *BMPR2* mutation was found (group A; Figure 7). Sixteen of the 31 patients with FPAH (52%) and 19 of the 78 IPAH patients (24%) carried a *BMPR2* mutation. No *BMPR2* mutation was found in 63

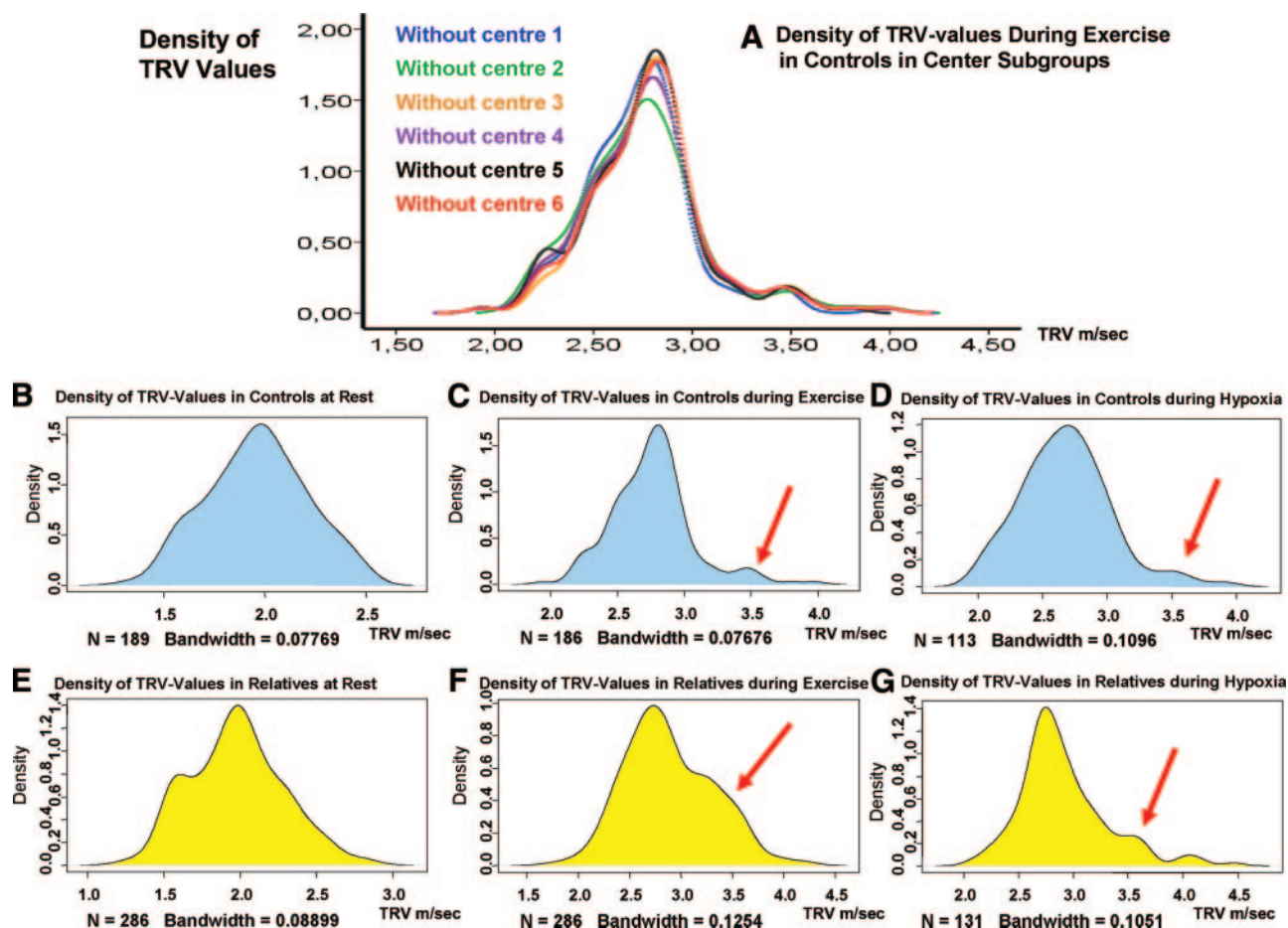


Figure 3. Density of TRV values in healthy control subjects (B through D) and relatives of IPAH patients (E through G). In the control subjects at rest (B), TRV values are normally distributed without a second peak of maximal values. During exercise (C) and during prolonged hypoxia (D), the density of TRV values is at least bimodal, with a second peak at ≈ 3.5 m/s representing the subgroup of subjects (10%) with hypertensive TRV response to exercise and hypoxia. Among the group of relatives (F and G), the second peak of values is not separated and has a larger area under the curve because of the higher proportion of subjects with hypertensive TRV response to exercise (F) or hypoxia (G). The calculated density profile in center subgroups is shown (A). Arrows delineate the second peaks of higher TRV values.

relatives of the *BMPR2*-positive index patients (group B) and 74 index patients and their relatives (group C; $n=197$). In 15 relatives, no genetic testing was performed. Mean maximal TRV during exercise was significantly higher in relatives with *BMPR2* mutation ($n=16$; 3.05 ± 0.41 m/s) than in relatives without *BMPR2* mutation ($n=260$; 2.87 ± 0.43 m/s; $P=0.029$). The group of relatives with *BMPR2* mutation (group A) had a significantly higher proportion of HR subjects (50.0%) than relatives without mutation (29.6%; 95% CI, 24.1 to 35.6; group B+C; $P=0.019$; Figure 7). The proportion of HR between the *BMPR2* mutation-positive (group A, 50.0%; 95% CI, 24.7 to 75.4) and *BMPR2* mutation-negative relatives (group B, 28.6%; 95% CI, 17.9 to 41.4) of *BMPR2* mutation-positive index patients also was significantly different ($P=0.045$). Mean TRV at rest (group A [$n=16$], 2.0 ± 0.25 m/s; group B+C [$n=260$], 1.92 ± 0.31 m/s; $P=0.7$) and mean maximal TRV during hypoxia (group A [$n=12$], 2.97 ± 0.65 m/s; group B+C [$n=121$], 2.90 ± 0.39 m/s; $P=0.71$) were not significantly different between the relatives of group A compared with those in group B+C.

Of the 291 relatives receiving echocardiography assessment, 71% were related to the index patient in the first degree

and 29% in the second degree. Nine percent of both the first- and second-degree relatives carried a *BMPR2* mutation. Relatives of *BMPR2*-positive index patients did not show a significantly different proportion of first- and second-degree relatives between the different groups (group A versus B; Figure 7). The group of *BMPR2*-negative relatives ($n=63$) consisted of 58% first-degree and 42% second-degree relatives. In comparison, the group of *BMPR2*-positive relatives ($n=16$) consisted of 69% first-degree and 31% second-degree relatives.

Discussion

This is the first prospective multicenter study devoted to screening for exercise- and hypoxia-induced PH in relatives of IFPAH patients and to investigate the threshold of normal pulmonary artery pressures during exercise in a large collective of healthy control subjects. One main finding of this study is that relatives of patients with IFPAH have a significantly higher frequency of HR to exercise and to prolonged hypoxia than control subjects. The highest proportion of HR subjects was found in relatives who shared a *BMPR2* mutation with the index patients. The data of this study suggest that a hypertensive pulmonary artery pressure response to exercise and hypoxia is genetically determined with a familial clustering.

Table 3. TRV in Different Groups

Characteristics	TRV at Rest, m/s	Maximum TRV During Exercise, m/s	TRV During Hypoxia, m/s	Sao ₂ During Hypoxia		
				At 60 min, %	At 90 min, %	At 120 min, %
Sex (all subjects)						
Men	1.97±0.29	2.86±0.38	2.80±0.42
Women	1.97±0.28	2.81±0.40	2.82±0.38
Smoking (all subjects)						
Smokers	1.99±0.29	2.80±0.40	2.79±0.40
Nonsmokers	1.97±0.28	2.85±0.39	2.81±0.40
Group						
Controls	1.96±0.25	2.76±0.30*	2.68±0.35*
Relatives	1.98±0.31	2.89±0.43*	2.92±0.41*
Center (controls)						
Giessen	1.94±0.27	2.97±0.44	2.87±0.39
Heidelberg	1.85±0.30	2.90±0.35	2.66±0.39
Brussels	1.93±0.24	2.70±0.37	2.91±0.37
Bologna	1.99±0.29	2.85±0.32
Clamart	2.15±0.26	2.86±0.36
Warsaw	1.99±0.23	2.69±0.39
All subjects						
Giessen	76.0±7.7	74.3±8.6	76.3±8.5
Heidelberg	76.6±6.5	75.8±6.5	77.0±6.3
Brussels	74.9±5.8	73.5±6.3	72.4±6.2

Values are given as mean±SD. TRV values were compared between subgroups: men versus women ($P=NS$), smokers versus nonsmokers ($P=NS$), control subjects versus relatives ($*P<0.05$), and between centres (global $P<0.05$).

Hypertensive TRV Response and *BMPR2* Gene

To the best of our knowledge, this is the largest study so far of healthy control subjects and relatives of I/FPAH patients using echocardiography in 482 subjects during exercise and 244 subjects during prolonged hypoxia. Patients, their relatives, and control subjects were recruited in 6 European centers (5 countries) specializing in PH. Thus, the study population represents at least 3 different white populations. All

centers found a variation of pulmonary artery pressures in control subjects and in relatives of PAH patients, with a proportion of subjects showing a hypertensive TRV during exercise and prolonged hypoxia (HR) not yet described in a larger population. Previous studies in small study populations have shown that pulmonary artery pressures during exercise are greatly variable among normal young individuals, some of whom show little change and some show large increments,

Table 4. Mean TRV and PASP at Rest and During Different Stages of Exercise and Hypoxia in Family Members and Control Subjects

TVR	TVR per Protocol Set, m/s		PASP per Protocol Set, mm Hg	
	Relatives (n=291)	Controls (n=191)	Relatives (n=291)	Controls (n=191)
During exercise				
At rest	1.98±0.31	1.96±0.25	20.7±5.4	20.4±5.3
25 W	2.43±0.43	2.32±0.31	28.6±5.7	25.5±5.4
50 W	2.64±0.48	2.49±0.34	32.9±5.9	29.8±5.5
75 W	2.80±0.49	2.61±0.32	36.4±5.9	32.5±5.4
100 W	2.86±0.40	2.67±0.33	37.7±5.6	33.5±5.4
125 W	2.94±0.37	2.81±0.31	39.5±5.6	35.5±5.4
During hypoxia				
30 min	2.45±0.36	2.31±0.35	29.0±5.5	26.3±5.5
60 min	2.6±0.43	2.46±0.35	32.0±5.7	29.1±5.5
90 min	2.78±0.42	2.59±0.37	36.1±5.7	31.9±5.6
120 min	2.90±0.46	2.70±0.38	38.8±5.8	34.2±5.8

Values are given as mean±SD.

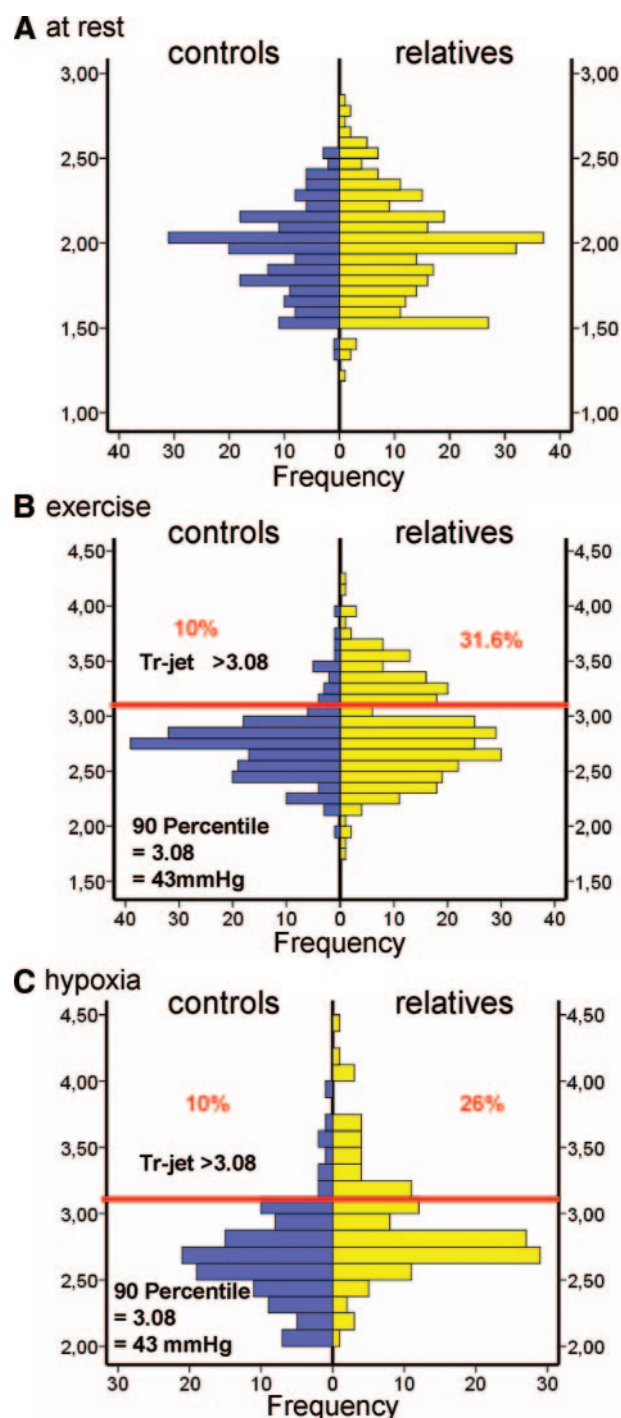


Figure 4. Histograms of TRV values at rest and during exercise and hypoxia in control subjects vs relatives. Bold horizontal line indicates the TRV cutoff value at 3.08 m/s separating subjects with hypertensive TRV response during exercise (B) and hypoxia (C).

depending on the exercise intensity.²⁵ In the present study, we found that these variations can be assigned to a bimodal distribution of maximal TRV values with a separate peak caused by the HR subgroup (Figure 3). HR subjects among relatives and control subjects had a distinct phenotype, with a 2- to 3-fold greater increase in mean TRV already during low levels of exercise (50 to 125 W) and/or during prolonged hypoxia compared with NR subjects. Some HR subjects reached peak

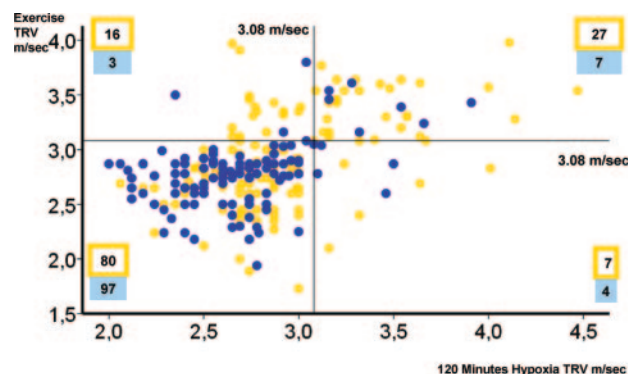


Figure 5. Scatterplot of maximum TRV values during hypoxia vs exercise in relatives (yellow) and control subjects (blue).

PASP values of up to 80 mm Hg. The finding of HR in relatives confirms the findings of a previous study in 2 families with FPAH.⁸ The mechanisms underlying the exaggerated pulmonary artery pressure response are currently unknown, with loss of pulmonary vasodilatory capacity, reduced distensibility, and inadequate/excessive vasoconstriction, as observed in subjects exposed to chronic hypoxia,²⁶ being discussed. The finding of a significantly higher proportion of HR subjects in I/FPAH relatives with *BMPR2* mutation compared with relatives without *BMPR2* mutation and compared with control subjects suggests that the HR phenotype—at least in I/FPAH—is genetically determined. However, the clinical implications of this finding are currently unknown. It is unlikely that the *BMPR2* mutations themselves cause HR for the following reasons. Not all subjects with a *BMPR2* mutation showed HR. Furthermore, relatives without *BMPR2* mutation also revealed a significantly higher proportion of HR subjects compared with control subjects (92 of 291 versus 19 of 191; $P < 0.0001$). Thus, HR may be distributed within the I/FPAH families as another genetic trait.

Our findings indicate that variation in TRV response to exercise and hypoxia is likely to be a “common” trait within the general population. It can be speculated that the “HR gene/s” may be necessary cofactor/s in an epistatic I/FPAH disease model supporting the previously suggested “second-hit” hypothesis.²⁷ To address these questions, long-term follow-up studies are necessary. The molecular basis of the hypertensive TRV response requires further investigation.

Hypertensive TRV Response to Hypoxia

Interestingly, TRV response to exercise correlated highly with the response to hypoxia. TRV values in HR subjects were in the same range as observed in individuals susceptible to high-altitude pulmonary edema.¹⁹ Thus, the question arises whether HR subjects in general are prone to develop high-altitude pulmonary edema and whether subjects susceptible to high-altitude pulmonary edema carry the same genetic disposition (HR gene/s).

Threshold of Normal TRV in Healthy Control Subjects

Traditionally it has been assumed that $TRV \leq 2.5$ m/s, corresponding to $PASP \leq 35$ mm Hg, during exercise represents the upper limit in healthy persons.^{20,28,29} However, no clear guidelines that distinguish normal from pathological

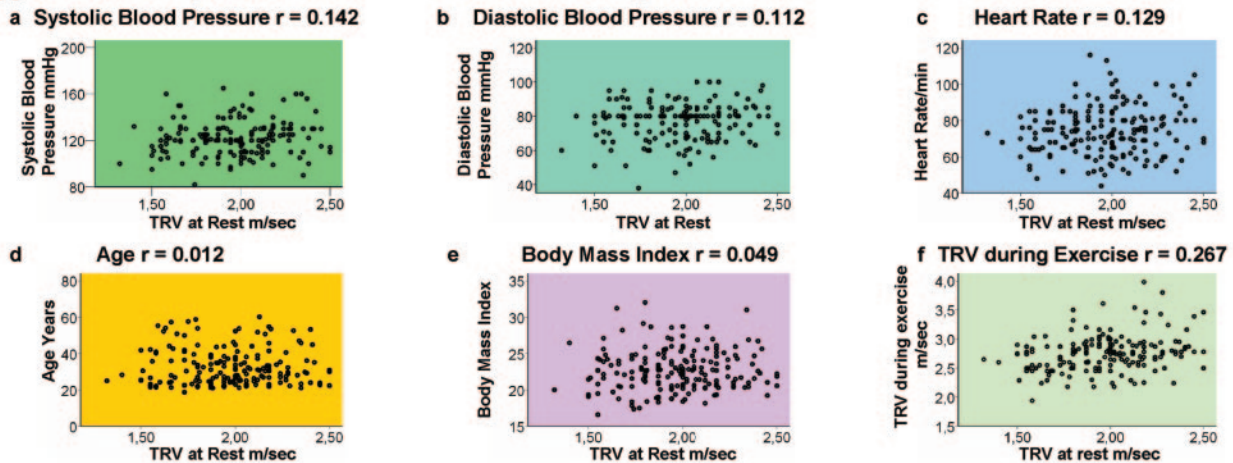
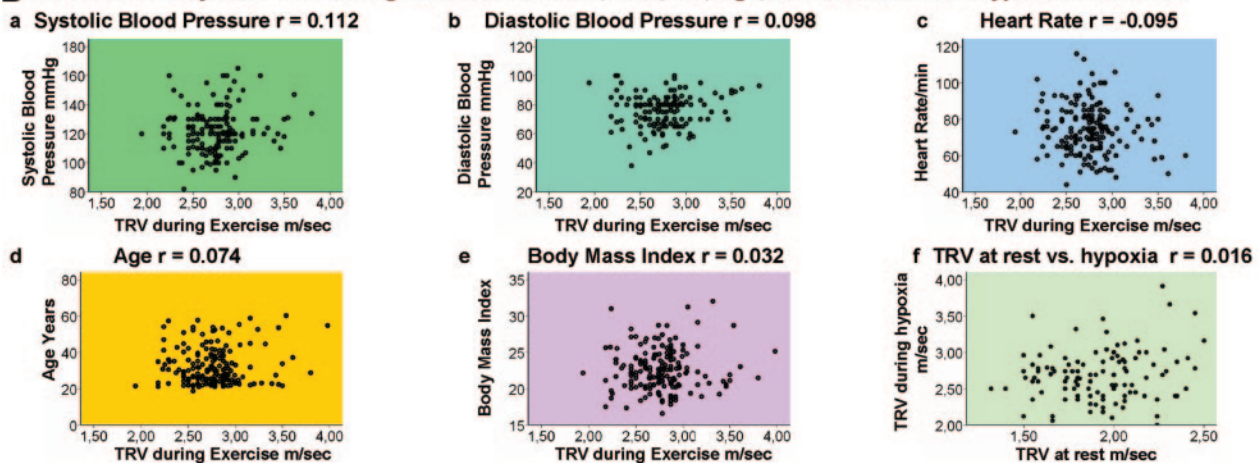
A Correlation Analysis of TRV at Rest with SBP, DBP, HR, Age, BMI and TRV exercise in Controls: Correlation coefficient r **B Correlation Analysis of TRV during Exercise with SBP, DBP, HR, Age, BMI and at rest vs. hypoxia in Controls**

Figure 6. Scatterplots demonstrating the correlation of TRV values (A) at rest and (B) during exercise in control subjects with the baseline variables age, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate frequency (HF), sex, and TRV values during exercise.

pulmonary artery pressure levels are available, particularly for Doppler echocardiography during exercise. The cutoff TRV value of 3.08 m/s (corresponding to 43 mm Hg PASP) defined in this study and separating NR from HR subjects was determined by measurements in a large number of healthy control subjects; 3.08 m/s was identical to the value obtained by density analysis and close to the mean peak value

plus 1 SD (3.06-m/s TRV=42.4-mm Hg PASP). Furthermore, this threshold is very consistent with previous invasive³⁰ and noninvasive^{15,31,32} studies that demonstrated mild increases in pulmonary arterial pressure with exercise in a normal population. However, this cutoff level might not be adequate in subjects with >60 years of age or at higher workloads (>150 W) and for athletes, TRV and PASP can

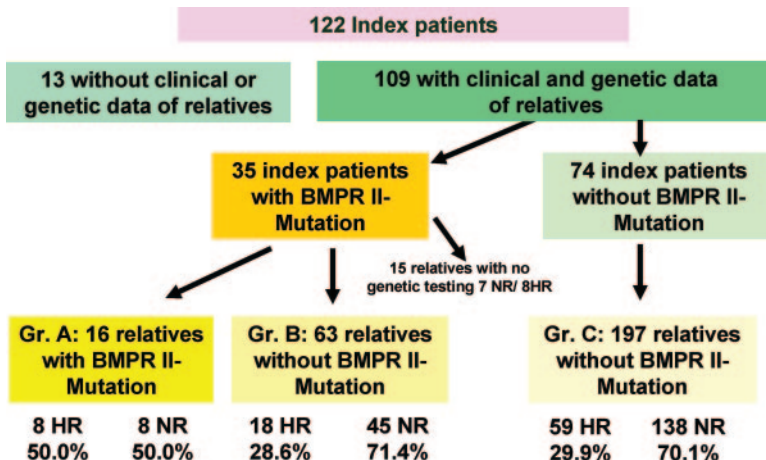


Figure 7. Genetic results. Flowchart of enrolled patients and relatives. Relatives carrying a *BMPR2* mutation had a hypertensive TRV significantly more often during exercise and/or hypoxia (HR) than individuals without a *BMPR2* mutation.

exceed 3.08 m/s and 43 mm Hg, respectively.¹⁵ We used a fixed right atrial pressure estimate of 5 mm Hg if the inferior vena cava was <20 mm. This may slightly underestimate or overestimate the true values, but given the consistency of this value between groups, it did not likely influence the results.

Correlation of TRV Values With Baseline Variables

Another major finding of this study is that TRV in control subjects at rest was not related to age, sex, systolic or diastolic blood pressure, and body mass index as suggested by McQuillan et al.³³ McQuillan et al evaluated PASP retrospectively from a database that did not explicitly exclude patients with coronary artery disease and/or pulmonary diseases. In our cohort, the TRV values were obtained prospectively, and subjects with systemic hypertension or other cardiopulmonary diseases were excluded. Nevertheless, we cannot exclude that other populations at an older age may present different results.

Clinical Implications

This study provides a basis for assessment of exercise- and hypoxia-induced PH. The clinical significance of a hypertensive pulmonary artery pressure response to exercise and hypoxia, however, is currently unsettled. It may reflect a normal variation of pulmonary artery pressures without clinical significance, or it may be related to some limitation in exercise capability that is relevant only under stress conditions. However, it may also reflect a genetic trait associated with increased risk for the development of PH or high-altitude pulmonary edema. In particular, the putative risk of developing PH among “HR-positive” relatives of I/FPAH patients must be discussed very cautiously. HR is also seen in healthy control subjects, although at a lower percentage than in I/FPAH relatives. Moreover, even if HR should turn out to reflect an enhanced risk for manifest PH development, the number of individuals identified as HR will be much higher than the number of subjects who will eventually develop the disease because of incomplete penetrance or possible further contributing factors. Follow-up assessments in relatives of I/FPAH patients are mandatory to clarify whether the HR state indeed reflects an enhanced risk to develop manifest PH and at what percentage. This will provide a basis to decide whether stress echocardiography is an appropriate tool for early diagnosis of PH.

Although great effort was necessary to standardize the echocardiographic examination and to obtain high-quality TRV profiles, the study showed that this screening approach is applicable in clinical settings. The TRV/PASP assessments during exercise and hypoxia are practicable and safe noninvasive tests to identify HR with high sensitivity and specificity. However, the true reliability of stress Doppler echocardiography during exercise or hypoxemia is currently unknown. The sensitivity of hypoxic and exercise testing is comparable and may even be improved further when both are combined. TRV/PASP is easier to assess during hypoxia than during exercise because the subjects do not move during the examination, and in most subjects, the tricuspid regurgitation profile is even more accentuated during hypoxia than at rest in normoxia or during

exercise. Nevertheless, it is not clear whether 1 of the 2 methods should be preferred for provocation of the HR.

Conclusions

Pulmonary hypertensive response to exercise and hypoxia in I/FPAH relatives appears in part to be a genetic trait with familial clustering, being correlated with but not caused by a *BMPT2* mutation. The clinical meaning of a hypertensive pulmonary artery pressure response to exercise and hypoxia remains unclear and should be addressed in long-term follow-up studies.

Acknowledgments

We wish to gratefully thank all patients, their family members, and the control subjects who participated in this study and the patients' self-care organization, pulmonale hypertonie einiger Verein for their great support. We thank Jeanette Röder for her support in the statistical analysis and Justyna Szamalek, PhD, for her work in the genetic assessment. We thank Peter Bärtsch and his team for allowing the group in Heidelberg to use the hypoxic chamber of the Institute of Sports Medicine.

Source of Funding

This study was funded by a grant of the European Union, fifth framework program, Disposition to PPH, QLGI-CT-2002-01116.

Disclosures

None.

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CLINICAL PERSPECTIVE

This is the first prospective multicenter study with Doppler stress echocardiography devoted to screening for exercise- and hypoxia-induced pulmonary hypertension in relatives of idiopathic or familial pulmonary arterial hypertension patients and to investigate the threshold of normal pulmonary artery pressures during exercise in a large collective of healthy control subjects. One main finding of this study is that relatives of patients with idiopathic or familial pulmonary arterial hypertension have a significantly higher frequency of a hypertensive tricuspid regurgitation velocity response to exercise and to prolonged hypoxia than control subjects. The highest proportion of subjects with hypertensive tricuspid regurgitation velocity response was found in relatives who shared a *BMPR2* mutation with the index patients. The data in this study suggest that a hypertensive pulmonary artery pressure response to exercise and hypoxia is genetically determined with familial clustering. The clinical significance of hypertensive pulmonary artery pressure response to exercise and hypoxia, however, is currently unsettled. It may reflect a normal variation in pulmonary artery pressures without clinical significance, or it may be related to some limitation in exercise capability that is relevant only under stress conditions. However, it may also reflect a genetic trait associated with increased risk for the development of pulmonary hypertension or high-altitude pulmonary edema. Although great effort was necessary to standardize the echocardiographic examination and to obtain high-quality tricuspid regurgitation velocity profiles, the study showed that this screening approach is applicable in clinical settings. Follow-up assessments in relatives of idiopathic or familial pulmonary arterial hypertension patients may provide a basis to decide whether stress echocardiography is an appropriate tool for early diagnosis of pulmonary arterial hypertension.